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Production and Emission of Volatile Compounds by Petal Cells

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Addendum to:

Both the adaxial and abaxial epidermal layers of the rose petal emit volatile scent compounds Véronique Bergougnoux, Jean-Claude Caissard, Frédéric Jullien, Jean-Louis Magnard, Gabriel Scalliet, J. Mark Cock, Philippe Hugueney, Sylvie Baudino. Planta 2007

KEY WORDS

Floral scent; Petal epidermis; *Rosa*; Terpenes; Volatiles

ABSTRACT

We localized the tissues and cells that contribute to scent biosynthesis in scented and non-scented *Rosa* x *hybrida* cultivars as part of a detailed cytological analysis of the rose petal. Adaxial petal epidermal cells have a typical conical, papillate shape whereas abaxial petal epidermal cells are flat. Using two different techniques, solid/liquid phase extraction and headspace collection of volatiles, we showed that, in roses, both epidermal layers are capable of producing and emitting scent volatiles, despite the different morphologies of the cells of these two tissues. Moreover, OOMT, an enzyme involved in scent molecule biosynthesis, was localized in both epidermal layers. These results are discussed in view of results found in others species such as *Antirrhinum majus*, where it has been shown that the adaxial epidermis is the preferential site of scent production and emission.

TEXT

Many plant species produce volatile compounds and these molecules serve a range of purposes. For example, compounds that are emitted from leaves are generally required for the defence of the plant against insect predators. On the other hand, floral compounds attract beneficial insects, leading to pollination of the flower. In leaves, scent compounds are very often synthesised in specialized cells grouped in structures termed trichomes or secretory glands. In many flowers, it is well documented that floral fragrance is produced by the corolla,[1] although other flower parts, such as the stamens in *Ranunculus acris*,[2] sometimes play an important role in fragrance emission. In some flowers, in particular those belonging to the Orchidaceae family, scent is emitted by specialized areas of the petal, which have been termed osmophores by Vogel.[3] However, in most flowers, when petals produce scent, it is thought to be emitted by all the cells of the petal in a diffusive manner.[4] In many flowers,

such as roses, the adaxial petal epidermal cells have a conical-papillate shape whereas the cells of the abaxial epidermis are flat (Fig.1).[5] The shape of these conical cells is controlled by a Myb-factor named MIXTA in *Antirrhinum majus*[6] and their shape has been shown to play a role in the diffusion of light, thereby enhancing the attractiveness of the flower.[7] Flowers of the *mixta* mutant have flat adaxial petal epidermal cells that reflect less light[8] and as a consequence attract less insects[9]. Along the same lines, Kolosova et al.[10] demonstrated that S-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase (BAMT), an enzyme involved in scent biosynthesis, was localized in the conical cells of the inner epidermal layer and to a much lesser extent, in the cells of the outer epidermis of the lobes of snapdragon petals. On the basis of these latter observations, some authors have proposed that the papillate cell shape could enhance the diffusion of scent molecules or influence its directionality and be of adaptive significance not only by enhancing light reflection but also by enhancing scent production[11, 12].

To test the hypothesis that the adaxial epidermis is a privileged site for the production and emission of scent, we chose a highly scented flower, the rose. Contrary to what was expected, we found that both the adaxial and abaxial epidermal layers of the petal were sites of scent production and emission. We were able to show that NaDi reagent stained purple droplets in both epidermal layers of the rose petal, indicating that they both contain terpenes. Several different techniques, including the analysis of epidermal peels and epidermal layer-specific headspace analysis failed to detect a strong difference between the production and emission of scent in the two epidermal layers. Moreover, the detection of OOMT protein, an enzyme involved in 3,5-dimethoxytoluene production, in both the abaxial and adaxial epidermis, indicated that biosynthesis of at least some phenolic scent compounds occurs in both tissues. It will be interesting to extend this approach using *in situ* hybridization or immunolocalization

to determine whether other pathways such as terpene metabolism are also active in the abaxial epidermis.

It is striking to note that in *Clarkia breweri*, which has actinomorphic flowers like the rose, expression of the S-adenosyl-L-methionine:(iso) eugenol O-methyltransferase (IEMT) gene seems to occur in both epidermal layers.[13] *A. majus* flowers have a different structure, they are highly zygomorphic with a flower shape that is adapted for bee pollination and includes specialized cell types in different parts of the flower (the lobes and the tube). To determine whether emission of scent in highly specialized flowers such as *A. majus* is linked to cell shape, it would be very useful to know whether *mixta* mutant flowers which have flat epidermal cells are impaired in their capacity to emit scent. However, the explanation may not be as simple. A recent study of the synthesis and emission of methyl benzoate showed that in *Nicotiana suaveolens*, as in the rose, both epidermal layers of the petal lobes are involved in scent production, whereas in *Stephanotis floribunda*, SAMT, another enzyme involved scent biosynthesis, is localized only in the adaxial epidermis and subepidermal regions of the flower petal lobes.[14] It is intriguing to note that *N. suaveolens* has bullate to rugose epidermal cell layers on both sides of the petal whereas *S. floribunda* has tight flat to bullate epidermal cells. The reasons for the differences in the potential for scent emission of the two petal epidermal layers in the rose and other species are not known. However, our results and a survey of the literature clearly indicate that, in petals, epidermal cells may have diverse shapes and that the shape of the cells is not necessarily a reliable indicator of the secretory potential of those cells. It will be interesting to see whether common structural features and/or molecular factors are responsible for the differences between these various cell types.

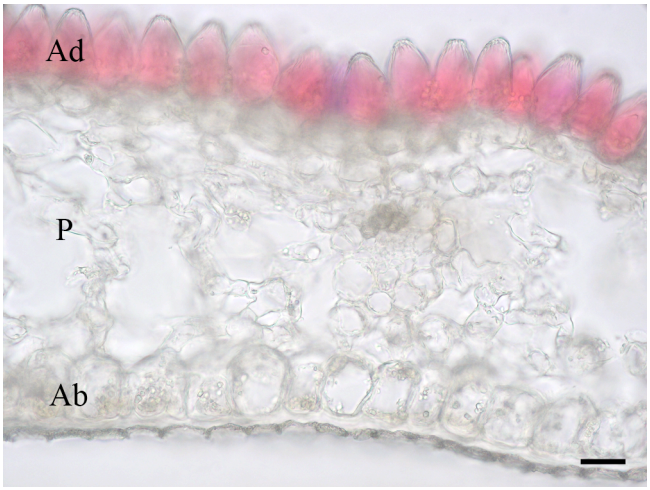

Figure 1. Hand-made cross-section of *Rosa* x *hybrida* petal; Ad, adaxial epidermis; Ab, abaxial epidermis; P, spongy parenchyma. Bar, 20 µm.

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